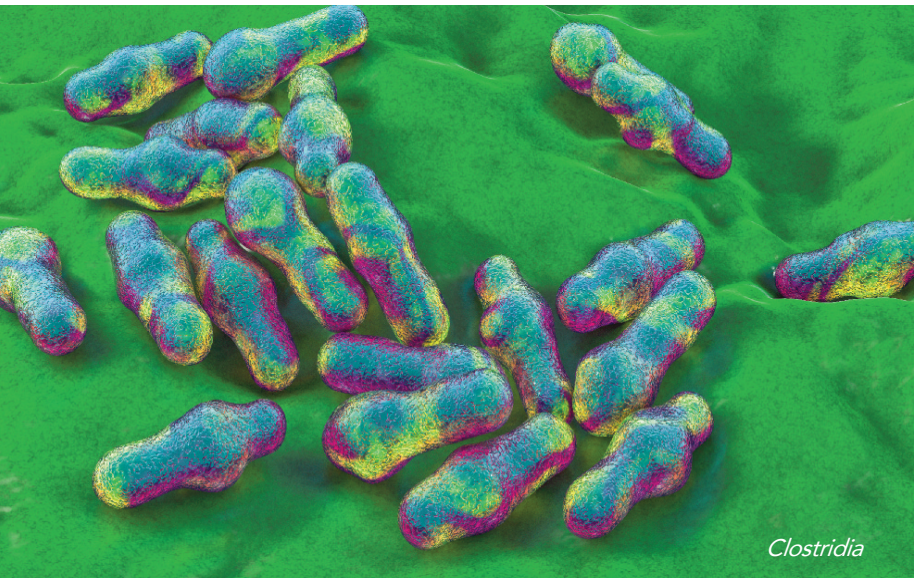
Benefits of *Lactobacillus acidophilus* D2/CSL (CECT 4529) as a feed additive for cats and dogs

SACCO system

Introduction

The intestinal microbiota of animals constitutes a complex ecosystem, which strongly influences normal intestinal function and preserves the animal's well-being. Moreover, **in synergy with the gut-associated lymphoid tissue (GALT), the intestinal microbiota hampers the invasion of exogenous microorganisms** (1,2). This process is known as "competitive exclusion". Microbial communities change over time due to physiological and environmental causes (e.g., aging, change in diet, stress, infections, antibiotic treatments) (3).



Clostridia

Mild dysbiosis can affect animals' health by modifying fecal characteristics or increasing fat in those predisposed to obesity (4). Furthermore, **the loss of anti-inflammatory bacteria and the increase of pro-inflammatory ones in the gut are associated with inflammatory responses and metabolic alterations, including the excessive development of adipose tissue** (5). Alterations of fecal consistency in dogs can be determined by mild dysbiosis due to certain diets responsible for **increasing the fecal count of clostridia and reducing bifidobacteria** (6).

Lactobacilli in the gut

However, more severe dysbiosis can cause severe adverse effects on intestinal physiology, including acute and chronic gastrointestinal inflammation, atopic disease, and intestinal cancer (5). For example, chronic inflammatory bowel disease, in humans as well as dogs, can be associated with an increase in Proteobacteria (e.g., Escherichia coli) and a decrease in other phyla (e.g., Firmicutes) in the gut (6).

In this regard, the scientific community is interested in **identifying ways to modulate the microbiota to improve intestinal health**; natural approaches, such as daily administration of probiotic lactobacilli, with anti-inflammatory properties on the digestive mucosa, could represent a valuable option (7). **Lactobacillus species, such as *Lactobacillus acidophilus*, are resident in the gastrointestinal tract of healthy dogs and cats** (8). This species colonizes the animal's gastrointestinal tract immediately after birth and **compete against enterobacteria and other pro-inflammatory microorganisms** (9).

Nevertheless, the abundance of lactobacilli in the intestinal microbiota varies over time. The balance between lactobacilli and pathogenic bacteria (e.g., Clostridium perfringens and Escherichia coli) can be impaired and often occur in domesticated animals, resulting in soft or aqueous stools (10).

Lactobacillus acidophilus D2/CSL (CECT 4529)

In human and veterinary medicine, probiotic lactobacilli are widely used to manage various disorders related to dysbiosis, such as antibiotic-associated diarrhea and intestinal inflammation (11). In particular, **probiotics can suppress inflammation of the mucosa** and possibly **promote anti-obesity effects** (12). However, the beneficial effects of the probiotic are strain-specific and rely on the dose (calculated in CFU) and duration of treatment (13).

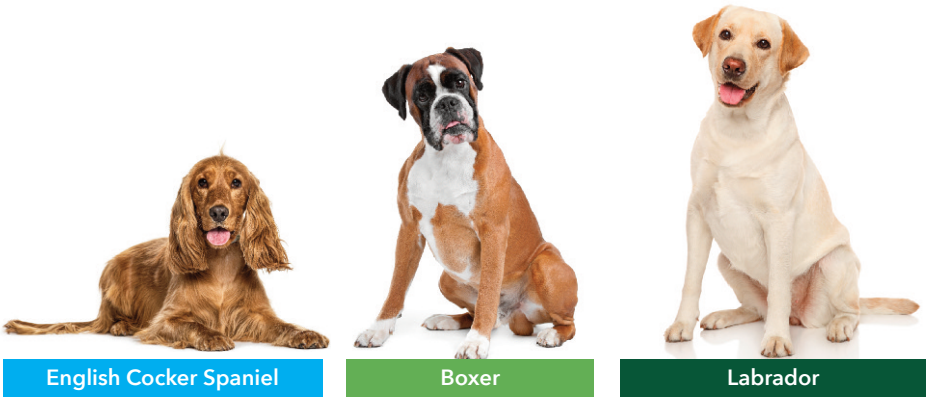
Lactobacillus acidophilus

Finally, animal health can lead to modulating the dosage or extending the probiotic treatment duration (14). **The probiotic strain *Lactobacillus acidophilus* D2/CSL (CECT 4529) was isolated by the Centro Sperimentale del Latte (CSL)** in 1985, with 18 other lactobacilli strains, from the intestine of a healthy adult chicken and owned exclusively by the CSL.

In particular, the *Lactobacillus acidophilus* D2/CSL stands out for the following:

- better resistance to low pH and bile acids;
- better ability to adhere to the intestinal epithelium and colonize the intestine;
- better "robustness" in the production phase (high vitality after freeze-drying).

***Lactobacillus acidophilus* D2/CSL beneficial effects on the gut have been investigated in healthy dogs and cats**



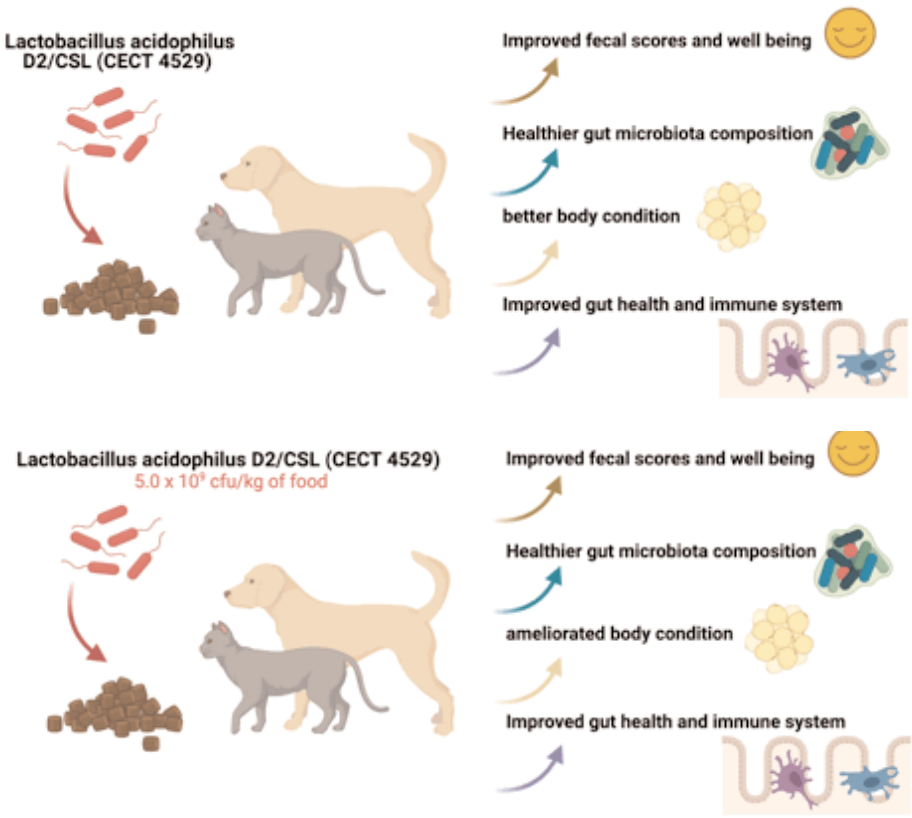
English Cocker Spaniel | Boxer | Labrador

Studies on dogs considered both males and females of three common breeds, with different body weights: English Cocker Spaniel (12-13 kg), Boxer (23-24 kg), and Labrador (30-31 kg).

Each test lasted 42 days, of which seven days of dietary adaptation and 35 days of treatment with *Lactobacillus acidophilus* D2/CSL (CECT 4529). The data collected during the tests (at T0, T14, T28, and T35 days) were related to the nutritional status of the animal, such as body weight (Kg), body condition score (BCS), evaluation of body fat mass, **as described from AAHA Nutritional Assessment Guidelines for Dogs and Cats, 2010** (15), as well as relating to various fecal parameters: fecal score (FS), fecal moisture (FM), fecal hardness (FH), and counting of fecal bacteria related to gut health. In these studies, *Lactobacillus acidophilus* D2/CSL (CECT 4529) was supplemented in food to a final concentration of 5.0 x 10^9 cfu/kg of food (recommended dosage). **Fecal parameters were considered the leading indicators of gut health of the animals**, and in particular:

Marelli and colleagues, assigned a commercial diet supplemented with *Lactobacillus acidophilus* D2/CSL (CECT 4529) to forty healthy adult boxer dogs (16). A control group received the same diet but without the probiotic. This study analyzed nutritional status (body weight, skinfold thickness, body condition score) and fecal quality parameters. In particular, skinfold measurements are meant to evaluate the nutritional status and quantify obesity. Similarly, the body condition score (BCS) identifies a direct method for evaluating nutritional status and ideal body condition with scores ranging from 1 to 9 (17). Here, the authors found no differences in body weight and skin thickness, yet confirmed that *Lactobacillus acidophilus* D2/CSL (CECT 4529) supplementation significantly improved fecal parameters and determined a significant difference in total *Escherichia coli* (*E. coli)* counts at 28 days. **In summary, this study shows that fecal consistency and microbiota composition improves by the inclusion of *Lactobacillus acidophilus* in dogs' diet.**

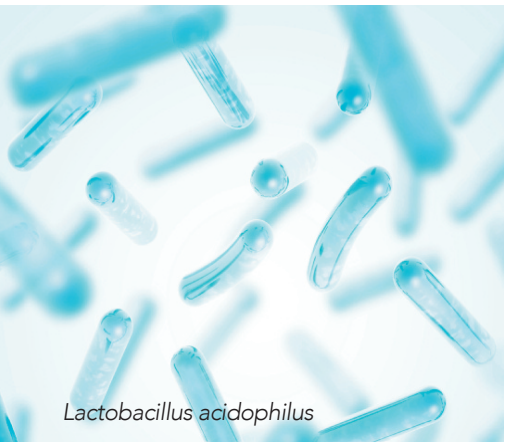
Body condition score improved gut health and fitness

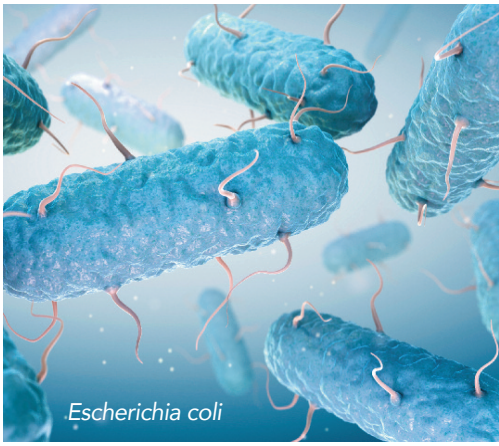


In a publication by Bruni et al., the effects of a diet integrated with the probiotic *Lactobacillus acidophilus* D2/CSL (CECT 4529) were evaluated in English Cocker Spaniel (ECS) and Labrador Retriever (LR) dogs (18). Thirty dogs were followed up for 35-days. This study assessed the benefits of *Lactobacillus acidophilus* D2/CSL (CECT 4529) supplementation on body weight, fecal parameters, and BCS. The BCS indicates a valid assessment of the body fat using a visual examination and palpation of the animal to which scores between 1 and 9 are assigned.

The researcher found that *Lactobacillus acidophilus* D2/CSL (CECT 4529) determined a significant skin thickness reduction throughout the trial. The improvements were also significant in the overall period for both dog breeds in the fecal characteristics (FM and FS). Besides, the BCS of dogs belonging to both breeds remained stable for the whole duration of the study in the treatment group, showing good maintenance of these dogs' nutritional conditions prone to overweight. It is important to point out that this study was performed on healthy dogs with no recorded overweight. **This work shows that *Lactobacillus acidophilus* D2/CSL (CECT 4529) can prevent and potentially treat overweight, promoting a beneficial intestinal microbiota involved in the regulation of fat storage.**

The study on cats was carried out in 10 adult healthy cats giant Maine Coon breed by Fusi and colleagues (19). Accordingly, to other studies, the probiotic administration in the treatment group was carried out by adding the lyophilized powder product to the commercial food. The animals in the control group received the same diet but without the probiotic. **Cats administered with *Lactobacillus acidophilus* D2/CSL showed improved fecal quality parameters.** Treated cats did not display changes in body weight and BCS. However, positive effects have been recorded in terms of **increased fecal lactobacilli counts and reduction in fecal *E. coli* counts showing that probiotics have a protective effect on invasive bacteria species.**


Lactobacillus acidophilus


Escherichia coli

Taken together, these data show that probiotics can balance the intestinal microbiota, reducing the number of pro-inflammatory bacteria and increasing the population of beneficial lactic acid bacteria.

A restored and healthy intestinal microbiota could bring immunomodulatory and anti-inflammatory effects due to the beneficial interaction of probiotic bacteria with the gut associated immune cells.

In summary, dogs fed with ***Lactobacillus acidophilus* D2/CSL** showed a better body condition. No difference in BCS was found in the cat study. The recommended amount of probiotic improved both fecal score (FS) and fecal consistency (FC), meaning that following treatment, animals had healthier consistent and formed stools. Accordingly, feeding animals with ***Lactobacillus acidophilus* D2/CSL** significantly reduced, over the 35-day duration of the test, the fecal moisture rate. Finally, the animals taking the probiotic showed an increase in fecal lactobacilli and a reduction in fecal *E. coli*, however with not always statistically significant differences.

Conclusions

Variation in fecal water content usually leads to fecal volume changes, fluidity, and stool frequency in companion animals (20); however, the causes of soft stool in cats and dogs are unknown. Some claim that a shorter transit time reduces water and absorption of electrolytes capacity in the gut, resulting in softer feces.

Others believe that the absorption of water and electrolytes does not have a critical impact on fecal consistency. However, excessive fermentation could cause inflammation of the intestinal mucosa and softer stool.

In this regard, probiotics could modify the gut microbiota, strengthen the gut epithelial barrier, improve body condition score and modulate the immune system to convey gut health.


Lactobacillus acidophilus

Probiotics are defined by the World Health Organization (WHO) as live microorganisms, which, when administered in adequate amounts, confer a health benefit on the host (7).

The long-term studies presented herein bring evidence for a role of the probiotic ***Lactobacillus acidophilus*** **D2/CSL (CECT 4529)** as a positive modulator of the intestinal microbiota in dogs and cats, at the endorsed dosage. In summary, the probiotic ***Lactobacillus acidophilus*** **D2/CSL** can significantly influence the balance between pro-inflammatory bacteria (e.g., pathogenic clostridia, *E. coli*) and anti-inflammatory bacteria (lactobacilli); these changes can determine a gradual positive evolution of the intestinal health as displayed by improved fecal characteristics. Importantly, the researchers assessed the effects on the reduction of fat accumulation in healthy dogs highlighting the potential of ***Lactobacillus acidophilus*** **D2/CSL to modulate the cross-talk between fat metabolism and the microbiota**.

Long-term *Lactobacillus acidophilus* D2/CSL (CECT 4529) supplementation, at the recommended dosage, can improve the animal physiological digestive function, well-being and body condition score (reduced overweight).

TAKE-HOME MESSAGES

The suggested amount of *Lactobacillus acidophilus* D2/CSL (CECT 4529) carries the potential to:

- Stabilize the functions of the normal gut microbiota in dogs and cats
- Balancing the gut microbiota between pro-inflammatory bacteria (pathogenic clostridia, *E. coli*, etc.) and anti-inflammatory bacteria (lactobacilli);
- Promote a gradual positive evolution of the intestinal health
- Improve the normal digestive functions (humidity and fecal consistency)
- Regulate fat storage in overweight animals
- Benefit the general well-being status of the animal

References

1. Jandhyala SM, Talukdar R, Subramanyam C, Vuyyuru H, Sasikala M, Reddy DN. Role of the normal gut microbiota. World J Gastroenterol. 2015 Aug 7;21(29):8787–803.

2. Robinson CJ, Bohannan BJM, Young VB. From Structure to Function: the Ecology of Host-Associated Microbial Communities. Microbiol Mol Biol Rev. 2010 Sep;74(3):453–76.

3. Hawrelak JA, Myers SP. The causes of intestinal dysbiosis: a review. Altern Med Rev. 2004 Jun;9(2):180–97.

4. Wang S, Tang J, Sun T, Zheng X, Li J, Sun H, et al. Survival changes in patients with small cell lung cancer and disparities between different sexes, socioeconomic statuses and ages. Sci Rep. 2017 May 2;7(1):1339.

5. Redfern A, Suchodolski J, Jergens A. Role of the gastrointestinal microbiota in small animal health and disease. Vet Rec. 2017 Oct 7;181(14):370.

6. Rizzatti G, Lopetuso LR, Gibiino G, Binda C, Gasbarrini A. Proteobacteria: A Common Factor in Human Diseases [Internet]. Vol. 2017, BioMed Research International. Hindawi; 2017 [cited 2021 Feb 16]. p. e9351507. Available from: https://www.hindawi.com/journals/bmri/2017/9351507/

7. Mack DR. Probiotics. Can Fam Physician. 2005 Nov 10;51(11):1455–7.

8. Barko PC, McMichael MA, Swanson KS, Williams DA. The Gastrointestinal Microbiome: A Review. J Vet Intern Med. 2018 Jan;32(1):9–25.

9. Zhao RX, Sun JL, Mo HZ, Zhu YY. Analysis of functional properties of Lactobacillus acidophilus. World Journal of Microbiology and Biotechnology. 2007;23(2):195–200.

10. Weese JS, Staempfli HR, Prescott JF, Kruth SA, Greenwood SJ, Weese HE. The roles of Clostridium difficile and enterotoxigenic Clostridium perfringens in diarrhea in dogs. J Vet Intern Med. 2001 Aug;15(4):374–8.

11. Kainulainen V, Tang Y, Spillmann T, Kilpinen S, Reunanen J, Saris PEJ, et al. The canine isolate Lactobacillus acidophilus LAB20 adheres to intestinal epithelium and attenuates LPS-induced IL-8 secretion of enterocytes in vitro. BMC Microbiology. 2015 Jan 16;15:4.

12. Wang J, Tang H, Zhang C, Zhao Y, Derrien M, Rocher E, et al. Modulation of gut microbiota during probiotic-mediated attenuation of metabolic syndrome in high fat diet-fed mice. ISME J. 2015 Jan;9(1):1–15.

13. De Cesare A, Sirri F, Manfreda G, Moniaci P, Giardini A, Zampiga M, et al. Effect of dietary supplementation with Lactobacillus acidophilus D2/CSL (CECT 4529) on caecum microbioma and productive performance in broiler chickens. PLoS One. 2017;12(5):e0176309.

14. Gallazzi D, Giardini A, Mangiagalli GM, Marelli S, Ferrazzi V, Orsi C, et al. Effects of Lactobacillus acidophilus D2/CSL on laying hen performance. Italian Journal of Animal Science. 2008 Jan 1;7(1):27–37.

15. Nutritional Assessment Guidelines for Dogs and Cats (2010) AAHA [Internet]. [cited 2021 Feb 16]. Available from: https://www.aaha.org/aaha-guidelines/nutritional-assessment-configuration/nutritional-assessment-introduction/

16. Marelli SP, Fusi E, Giardini A, Martino PA, Polli M, Bruni N, et al. Effects of probiotic Lactobacillus acidophilus D2/CSL (CECT 4529) on the nutritional and health status of boxer dogs. Vet Rec. 2020 Aug 22;187(4):e28.

17. WSAVA Nutritional Assessment Guidelines Task Force Members. WSAVA nutritional assessment guidelines. J Feline Med Surg. 2011 Jul;13(7):516–25.

18. Bruni, Natascia, Martello, Elisa, Fusi E, Meineri, Giorgia, Giardini A. Study of faecal parameters and body condition in dogs with a diet supplemented with Lactobacillus acidophilus D2/CSL (CECT 4529),Italian Journal of Animal Science - X-MOL [Internet]. [cited 2021 Feb 16]. Available from: https://www.x-mol.com/paper/1283168155303854080

19. Fusi E, Rizzi R, Polli M, Cannas S, Giardini A, Bruni N, et al. Effects of Lactobacillus acidophilus D2/CSL (CECT 4529) supplementation on healthy cat performance. Open access. :6.

20. Rolfe VE, Adams CA, Butterwick RF, Batt RM. Relationship between faecal character and intestinal transit time in normal dogs and diet-sensitive dogs. J Small Anim Pract. 2002 Jul;43(7):290–4.